

09-9-28-BLST-LB-SI-QA

LABORATORY NOTEBOOK

Data Verification and
Validation Log Book for
Siemens SiCURE Performance
Evaluation

Kelsey Prihoda-Barstow 7

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- Sample Collection 09 SI 1
• Learned that tubs 4 and 5 could not be drained simultaneously and only one zooplankton sample was collected from tub 5.

- Sample Collection 09 SI 2 ^{09 SI 2 09}
• During fill the bay pump stopped running approximately 12 minutes into fill. Water that had been collected in tubs 4 and 6 was lost, therefore, a portion of the sample was collected after the bay pump began running again.

Water in Control Tank before pump shutdown = 12,050 gallons
Water in Control Tank after pump shutdown = 50,251 gallons
62,301 gallons

$(12,050 \text{ gal} / 62,301 \text{ gal}) 100\% = 19.3\%$ of sample lost.

Water in Treatment Tank before pump shutdown = 11,756 gallons
Water in Treatment Tank after pump shutdown = 49,803 gallons
61,559 gallons

$(11,756 \text{ gal} / 61,559 \text{ gal}) 100\% = 19.1\%$ of sample lost.

- Sample Collection 09 SI 3
• During discharge collected 3.8 m³ water into sample collection tubs. Samples from Treatment Tank were collected from tubs 4 and 5 only because Tub 6 and associated lines were not flushed prior to discharge. No water quality measurements from tubs.

- Sample Collection 09 SI 4 → Sample lines going to tubs were replaced.
• During discharge a small amount of very dirty water flowed into tub 4, and all samples were collected from tubs 5 and 6.
• Salinity (ppt) was higher during discharge than previous trials, and higher than post-treatment intake salinity.

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- Sample Collection 09 SI 5
• Variability seen in salinity measurements from treatment tubs during discharge. Treatment tub 4 and 5 = 1.1 ppt, but tub 6 = 0.5 ppt. Was water level too low to measure water quality in rinse/sonde bucket?

- Sample Collection 09 SI 6
• Again, variability seen in salinity measurements from treatment tubs during discharge. Tubs 4 and 5 = 0.8 ppt, but Tub 6 = 0.3 ppt. Could be due to low water level in rinse/sonde bucket.

30 September 2009 KMP

- Sample Collection 09 SI 7
• At the start of the fill, tub #4 began filling at ~20 gallons per minute, while Tubs #5 and #6 began filling at the goal rate of ~9.5 gallons per minute. The problem was corrected several minutes into the fill, and there was no effect on the volume of water collected in Tub #4. However, Tub #5 was sampled rather than Tub #4 to keep consistency between the pre- and post-treatment samples.

01 October 2009 KMP

- Sample Collection 09 SI 7 Discharge
• At the start of Treatment Tank #1 discharge, Tub #4 began filling at a faster rate than Tubs #5 and #6. This also occurred during the Control Tank #1 discharge, when Tub #3 began filling faster than Tubs #1 and #2. There was no effect on the volume of water collected in Tubs #4 and #1 and samples were collected according to the test plan.

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01 Oct. 09 (cont'd)

- Treatment and Control Tank agitation was conducted by an AMI intern (Tyler) who had never performed the agitation procedure before. Tyler did not have sufficient training to carry out this procedure and DMR observed and noted that the procedure was being carried out differently than previous trials.

- Zooplankton Analysis Data Verification

- Zooplankton sample analysis data was verified by checking data recorded on the "Zooplankton Identification Worksheet" and "Documentation of Live Zooplankton Found During Treatment Discharge" datasheets against the following SOP:

• GSI/SOP/LB/RA/SA/2 - Procedure for Zooplankton Sample Analysis (DRAFT)

- Findings were recorded on the Technical Systems Audit for each trial's fill and discharge.

05 Oct. 2009 KMP

- Whole Effluent Toxicity (WET) Test Data Verification

- WET testing data was verified by checking data recorded on the stock solution preparation, *C. dubia* WET test, *P. promelas* WET test, and *S. capricornutum* WET test data sheets against the following SOPs:

- GSI/SOP/BS/RA/RT/6 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*.
- GSI/SOP/BS/RA/RT/7 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Fathead Minnow (*Pimephales promelas*)

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05 Oct. 09 (cont'd)

- GSI/SOP/BS/RA/RT/8 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Green Alga (*Selenastrum capricornutum*).
- Findings were recording on the WET Testing Datasheets (QA section) and on the Technical Systems Audit for each trial's fill and discharge.

05 Oct. 09 KMP

- Continued with zooplankton sample analysis data verification for Trials 5-7. Findings were recorded on Technical Systems Audit for each trial's fill and discharge.

07 October 2009 KMP

- Began proofing WET Testing data entry for Trials 1, 3, 4, 5, 6, + 7. Due to the large amount of data, 10% of the entries will be proofed. If a significant amount of correction is needed on the 10% (randomly chosen), then the entire data set will be proofed.

C. dubia data proofing: $(8 \text{ trts}) \left(\frac{10 \text{ reps}}{\text{trt}} \right) = 80 \text{ reps} \therefore \text{proof } 8 \text{ entries}$
Entry = # adults + # young per test day

S. capricornutum data proofing: $(8 \text{ trts}) \left(\frac{4 \text{ reps}}{\text{trt}} \right) = 32 \text{ reps} \therefore \text{proof } 4 \text{ entries}$
Entry = # cells 4 entries per day

P. promelas data proofing: $(8 \text{ trts}) \left(\frac{4 \text{ reps}}{\text{trt}} \right) = 32 \text{ reps} \therefore \text{proof } 4 \text{ entries}$
Entry = # larvae + dry weight 4 entries per day

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08 October 2009 KMP -Continued WET Test Data Verification and Data Entry Proofing for Siemens Trials 6 and 7.			
13 October 2009 KMP -Started data verification and validation for Siemens Phytoplankton data. Data entered into the database by Euan and/or Lisa has been proofed by a second individual. Phytoplankton analysis data verification will be done by checking phytoplankton data sheets (scanned and posted on SharePoint site) against the following SOP: <ul style="list-style-type: none"> GSI/SOP/LB/RA/MA/1 - Procedure for Algae/Small Protozoan Sample Analysis. 			
-Findings from data verification are recorded on the Technical Systems Audit data sheets for each trial's fill and drain.			
14 October 2009 KMP -Continued phytoplankton data verification and validation. Will start with final verification trials (#3-#7) and end with preliminary trials (#1-2).			
16 October 2009 KMP -Continued phytoplankton data verification/validation. The diatom chain results are difficult to read on the data sheet due to the extremely large number of cells. Would it be possible to use a counter to total the cells as the sample is being analyzed?			
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19 October 2009 KMP -Continued phytoplankton data verification/validation. Left to review: Trials 6, 7, 1, and 2.			
20 October 2009 KMP -Continued zooplankton data verification/validation. Left to review: Trials 6 and 7 datasheets			
21 October 2009 KMP -Began microbial analysis data verification and validation for Siemens SiCURE Performance Evaluation. Microbial sample analysis data was verified by checking datasheets in binder #09-8-26-BLST-LB-SI-MICRO against the following SOPs: <ul style="list-style-type: none"> GSI/SOP/BS/RA/MA/1 - Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate for HPC method GSI/SOP/BS/RA/MA/3 - Procedure for the Detection and Enumeration of Enterococcus using Enterolert GSI/SOP/BS/RA/MA/4 - Procedure for the Detection and Enumeration of Total Coliforms and E. coli using IDEXX's Colilert DRAFT GSI SOP - Procedure for Colony Blot Preparation for the Enumeration of Culturable Vibrio cholerae 			
-Findings were recorded on the Technical Systems Audit Checklist for each trial's fill and discharge. <ul style="list-style-type: none"> -Started with test trials (#3-#7) and will finish with calibration trials (#1-#2). 			
23 October 2009 KMP -Continued microbial analysis data verification			
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23 Oct. 2009 (cont'd)
and validation for Trials 6, 7, 1 and 2.

26 October 2009 KNP
-Continued microbial sample analysis data verification and validation; for Trial 1 Discharge and Trial 2.

-Began completing any SOP Deviation Forms needed for Trials 1-2, 3-4. SOP deviations were noted on Technical Systems Audit Checklists for each Trials Fill and Discharge. For each SOP Deviation, a description of the deviation (SOP), description of impact on the study, description of corrective actions taken, and relevant staff member comments will be provided.

27 October 2009 KNP
-Continued completion of SOP Deviation Forms for Trials 5-7 and 1-2.

-Began completing any SOP Amendment Forms needed for Siemens SiCURE Performance Evaluation. An amendment is a permanent change to an SOP. The reason for the amendment, SOP sections affected, date implemented, and relevant staff member comments will be provided.

28 October 2009 KNP
-Continued completion of SOP Amendment Form for Siemens SiCURE Performance Evaluation.
-Completed all SOP Amendment Forms. All SOP Deviations and Amendments are listed on pages 9-13 of this log book.

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Great Ships Initiative

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GREAT SHIPS INITIATIVE (GSI) TEST PLAN DEVIATIONS AND STANDARD OPERATING PROCEDURE (SOP) DEVIATIONS/AMENDMENTS

SIEMENS SiCURE PERFORMANCE EVALUATION GSI RESEARCH, DEVELOPMENT, TESTING, AND EVALUATION FACILITY

SOP AMENDMENTS (TRIALS 1-7 DEVIATIONS)

- SOP No: GSI/SOP/LB/RA/SC/4 - Procedure for Microbial Sample Collection
 - Section "QA/QC", ¶3. Collect one field duplicate per treatment system being tested (or a minimum of 10% of total samples collected).
- SOP No: GSI/SOP/BS/RA/C/6 - Procedure for Analyzing Total Residual Chlorine Concentrations in Water
 - ¶6a.-e. Verify the accuracy of the 100 mg/L bleach solution using the potassium iodate verification standard. If the 100 mg/L bleach solution is not accurate (i.e., a difference of 3 mV from the verification standard), the concentration of the solution prepared from bleach will need to be modified.
- SOP Nos: GSI/SOP/BS/RA/MA/1 - Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate for HPC; GSI/SOP/BS/RA/MA/3 - Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert; GSI/SOP/BS/RA/MA/4 - Procedure for the Detection and Enumeration of Total Coliforms and *E. coli* using IDEXX's Colilert
 - Section "Sample Collection", ¶2. Inactivate or neutralize the active substance at the time of sample collection or when a defined exposure period has been reached (i.e., sodium thiosulfate to neutralize chlorine). Sterile vessels purchased from IDEXX provide enough sodium thiosulfate to neutralize up to 10 ppm chlorine.
- GSI/SOP/BS/RA/RT/6 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*
 - Section "Test Procedure", ¶3. Add 15 mL of the appropriate exposure solution to ten replicate 30 mL borosilicate glass beakers or disposable polystyrene cups.

TRIALS 1 AND 2 (CALIBRATION TRIALS) DEVIATIONS

Test Plan Deviations

- During 09-SI-1-Discharge, discovered that two sample collection tubs cannot be drained at the same time for zooplankton sample collection. A zooplankton sample was collected from Tub 5 instead of Tubs 4 and 5 for this reason.
- During 09-SI-2-Fill, the bay pump stopped running approximately 12 minutes into fill. Water that had been collected in sample collection Tubs 1, 4, and 6 was lost (19.3% of sample lost from control tubs and 19.1% of sample lost from treatment tubs). The remaining sample water was collected after the bay pump began running again.

SOP Deviations

- SOP No: GSI/SOP/BS/RA/MA/1 - Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate for HPC Method

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- a. Section "Sample Collection", ¶2. Inactive or neutralize the active substance at the time of sample collection or when a defined exposure period has been reached (i.e., sodium thiosulfate to neutralize chlorine).
2. SOP No: GSI/SOP/LB/RA/SA/1 – Procedure for Algae/Small Protozoan Sample Analysis
 - a. Section "QA/QC", ¶3. Ensure a second analyst counts at least 10% of samples to provide consistency and replicability of assessment methods and taxonomy.
3. SOP No: GSI/SOP/BS/RA/RT/6 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*
 - a. Section "Test Procedure", ¶11. Feed the test organisms daily. Food is added to freshly renewed exposure solutions immediately before or immediately after the adults are transferred. Each feeding consists of 0.1 mL Yeast-Cereal Leaves-Trout Chow suspension (YCT) and 0.1 mL *Selenastrum capricornutum* concentrate/15 mL exposure solution (to provide 2-2.3 x 10⁵ cells/mL).
4. SOP No: GSI/SOP/BS/RA/RT/7 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Fathead Minnow (*Pimephales promelas*).
 - a. Section "Test Procedure", ¶7. Feed the test organisms twice daily at 6-hour intervals. Larvae are fed approximately 0.1g of a concentrated solution of less than 24-hour old brine shrimp (*Artemia* spp.).
5. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. QA/QC ¶4. Ensure a second operator counts the algae cell concentration in at least 10 % of the test chambers.
6. SOP No: GSI/SOP/LB/RA/SA/2 – Procedure for Zooplankton Sample Analysis (DRAFT)
 - a. QA/QC ¶3. Ensure that a second analyst counts at least 10% of samples in duplicate to provide consistency and replicability of assessment methods and taxonomy. If an experimental trial has less than 10 samples, at least one sample from each trial should be analyzed in duplicate.

TRIAL 3 DEVIATIONS**Test Plan Deviations**

1. Discharge Test Plan – September 9, 2009
 - a. Sample collection tubs 4 and 5 were filled to 3.8 m³. No samples were collected from sample collection tub 6 because the tub and associated sample lines were not flushed prior to discharge. Therefore, all samples that were to be collected from Tub 6 were collected from Tub 4 (i.e., two subsamples of phytoplankton, microbes, and chemistry; and disinfection byproducts and whole effluent).
 - b. No water quality measurements (i.e., temperature, pH, and salinity) were taken from water in sample collection tubs 1, 4, or 5.

SOP Deviations

1. SOP No: GSI/SOP/LB/RA/SA/2 – Procedure for Zooplankton Sample Analysis (DRAFT)
 - a. Section "QA/QC", ¶3. The QA analysis is performed on one of the rotifer subsamples/slides and one of the crustacean subsamples/counting chambers for the selected QA sample.
2. SOP No: GSI/SOP/BS/RA/MA/3 and /4 – Procedure for the Detection and Enumeration of *Enterococcus* using Enterolert (and Total Coliforms and *E. coli* using IDEXX's Colilert)

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- a. MA/3 – Section "Quanti-Tray Enumeration Procedure", ¶12. Read results between 24-28 hours...Note: Enterolert results are definitive at 24-28 hours.
- b. MA/4 – Section "Quanti-Tray Enumeration Procedure", ¶11. Results are definitive at 24-28 hours.
3. SOP No: GSI/SOP/RDTE/SA/M/3 – Procedure for the Colony Blot Preparation for the Enumeration of Culturable *Vibrio cholerae* (DRAFT)
 - a. Section "RNA Colony Blot Preparation", ¶14-21. Dry membranes for 10 min. at 36°C. Using a handheld UV light, hold light 1.5 inches above the membrane and move back and forth across the membrane for 1 min. Bake membranes for 15 min. at 70°C.
4. SOP No: GSI/SOP/BS/RA/RT/6 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*
 - a. Section "Test Procedure", ¶11. Feed the test organisms daily. Food is added to freshly renewed exposure solutions immediately before or immediately after the adults are transferred. Each feeding consists of 0.1 mL Yeast-Cereal Leaves-Trout Chow suspension (YCT) and 0.1 mL *Selenastrum capricornutum* concentrate/15 mL exposure solution (to provide 2-2.3 x 10⁵ cells/mL).
5. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "Test Procedure", ¶5. Ensure that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/ml (± 10%) in the test flasks.
6. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "QA/QC", ¶4. Ensure a second operator counts the algae cell concentration in at least 10 % of the test chambers.
7. SOP No: GSI/SOP/BS/RA/RT/7 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Fathead Minnow (*Pimephales promelas*)
 - a. Section "Test Procedure", ¶7. Feed the test organisms twice daily at 6-hour intervals.

TRIAL 4 DEVIATIONS**Test Plan Deviations**

1. During discharge, a small amount of very dark-colored water (rusty) flowed into sample collection Tub 4; therefore, all samples from treatment discharge were collected from Tubs 5 and 6.

SOP Deviations

1. SOP No: GSI/SOP/LB/RA/SA/2 – Procedure for Zooplankton Sample Analysis (DRAFT)
 - a. Section B.i., ¶d. To ensure accuracy in counting, there should be 150 organisms ideally, but no more than 200 organisms present in a single 1 mL subsample.
2. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "Test Procedure", ¶5. Ensure that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/ml (± 10%) in the test flasks.
3. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)

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- a. Section "QA/QC", ¶4. Ensure a second operator counts the algae cell concentration in at least 10 % of the test chambers.
- 4. SOP No: GSI/SOP/BS/RA/RT/6 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*
 - a. ¶5. Acclimate neonate *C. dubia* for approximately 24 hours at test temperature by transferring <24 hour old neonates from brood boards to 50 % culture water (i.e., hard reconstituted water)/50 % test water.

TRIAL 5 DEVIATIONS

SOP Deviations

1. SOP No: GSI/SOP/BS/RA/MA/3 – Procedure for the Detection and Enumeration of Enterococcus using Enterolert
 - a. Section "Quanti-Tray Enumeration Procedure", ¶2. Run a control blank with each set of samples using the same water type.
2. SOP No: GSI/SOP/BS/RA/MA/4 – Procedure for the Detection and Enumeration of Total Coliforms and *E. coli* using IDEXX's Colilert
 - a. Section "Quanti-Tray Enumeration Procedure", ¶2. With each set of samples, run a control blank of the same water sample.
3. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "Test Procedure", ¶5. Ensure that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/ml (\pm 10%) in the test flasks.
4. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "QA/QC", ¶4. Ensure a second operator counts the algae cell concentration in at least 10 % of the test chambers.
5. SOP No: GSI/SOP/BS/RA/RT/6 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to *Ceriodaphnia dubia*
 - a. ¶5. Acclimate neonate *C. dubia* for approximately 24 hours at test temperature by transferring <24 hour old neonates from brood boards to 50 % culture water (i.e., hard reconstituted water)/50 % test water.

TRIAL 6 DEVIATIONS

SOP Deviations

1. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section, "Test Procedure", ¶5. Ensure that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/ml (\pm 10%) in the test flasks.
2. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "QA/QC", ¶4. Ensure a second operator counts the algae cell concentration in at least 10 % of the test chambers.

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TRIAL 7 DEVIATIONS

Test Plan Deviations

1. During control and treatment tank fill, sample collection Tub 4 began filling at approximately 20 gallons per minute, while Tubs 5 and 6 began filling at the desired rate of approximately 9.5 gallons per minute. The flow rate into Tub 4 was corrected several minutes into the fill, and there was no effect on the volume of water collected in Tub 4. To maintain consistency between the pre- and post-treatment samples, Tub 5 was sampled rather than Tub 4 for pre-treatment.

SOP Deviations

1. SOP No: GSI/SOP/LB/RA/SA/1 – Procedure for Algae/Small Protozoan Sample Analysis
 - a. Section "QA/QC", ¶3. Ensure a second analyst counts at least 10% of samples to provide consistency and replicability of assessment methods and taxonomy.
2. SOP No: GSI/SOP/LB/RA/SA/2 – Procedure for Zooplankton Sample Analysis (DRAFT)
 - a. Section B.i., ¶1d. To ensure accuracy in counting, there should be 150 organisms ideally, but no more than 200 organisms present in a single 1 mL subsample.
 - b. Section B.ii, ¶1c. Use this density estimate to determine whether the original sample will need to be concentrated or diluted to obtain approximately 150-200 organisms per subsample.
3. SOP No: GSI/SOP/BS/RA/MA/1 – Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate for HPC Method
 - a. Section "Sample Collection", ¶4. Analyze samples within 4-6 hours of collection. In exceptional circumstances, i.e., if there is a delay, store samples in a refrigerator at 2-8°C for a maximum of 24 hours before beginning analysis.
4. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "Test Procedure", ¶5. Ensure that each milliliter of inoculum contains enough cells to provide an initial cell density of approximately 10,000 cells/ml (\pm 10%) in the test flasks.
5. SOP No: GSI/SOP/BS/RA/RT/8 – Procedure for Assessing Chronic Residual Toxicity of a Ballast Water Treatment System to the Green Alga (*Selenastrum capricornutum*; DRAFT)
 - a. Section "QA/QC", ¶4. Ensure a second operator counts the algae cell concentration in at least 10 % of the test chambers.

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